Characterization of C. jejuni strains representing broiler meat chain

Kudirkiené, Eglé; Brøndsted, Lone; Stabler, Richard; Cohn, Marianne Thorup; Šernien, Loreta; Malakauskas, Mindaugas

Publication date:
2010

Document version
Publisher's PDF, also known as Version of record

The EC Review Meeting and

4th General Meeting

22-23 March 2010

Copenhagen, Denmark

Characterization of *C. jejuni* strains representing broiler meat chain

Eglė Kudirkienė[1]*, Lone Brøndsted[2], Richard Stabler[3], Marianne Thorup Cohn[2], Loreta Šerniené[1], Mindaugas Malakauskas[1]

[1] Lithuanian Veterinary Academy, Department of Food Safety and Animal Hygiene, Lithuania

[2] University of Copenhagen, Department of Veterinary Disease Biology, Denmark

[3] London School of Hygiene and Tropical Medicine, Department of Infectious & Tropical Diseases, UK

INTRODUCTION

Broiler meat may be contaminated during slaughter at the evisceration step or when the meat comes into contact with equipment and scalding water infected with *C. jejuni*. Another important source of contamination is *C. jejuni* strains remaining in slaughterhouse after disinfection. Several studies have indicated that only some strains among those that enter the slaughterhouse can survive all processing and result in contamination of the final product at the end of slaughter. We hypothesize that characterization of the *C. jejuni* strains obtained along the entire meat production chain will provide us an understanding of the survival mechanisms of *C. jejuni* in the adverse environment of the slaughterhouse and will serve as a basis to develop strategies to control this food-borne pathogen.

CONCLUSIONS

Results of the present study show that broiler meat contamination with *C. jejuni* and *C. jejuni* survival in the environment is dependant on both strains adaptation to low temperatures and ability to form biofilm.

The adaptation to ambient temperature shared by clinical and persistent broiler cloacae strain indicate that this might be specific property required for strains persistence and circulation in the environment.

More detailed analysis of the gene content between strains belonging to the same phylogenetic group and sharing the same phenotypic features as demonstrated in the present study would be helpful to identify the regions or genes groups contributing to adaptive properties of the strains.

AIM

The aim of our study was to compare survival and adaptation abilities of 10 different strains of *C. jejuni* collected during slaughtering of one broiler flock. As controls, two additional strains: clinical and persistent broiler cloacae strain, were included. The effect of genotype, origin and phylogeny on strain phenotype was determined.

METHODS

12 strains of *C. jejuni* representing distinct *flaA* RFLP genotypes obtained from broiler meat chain were tested. All strains were grouped by there source of isolation (Table 1). Stationary phase bacterial cultures grown in BHI were adjusted to 0.2 at OD_{600} and inoculated into sterile chicken juice prepared as described by Birk et. al. (2004) 1:10 and incubated for 30 days at 5°C, 18 days at 20°C microaerobically and for 35 days and 3 hours at -18°C and 48°C in atmospheric air, respectively. Samples kept in chicken juice were withdrawn every third day when kept at 5°C and 20°C, every five days when kept at -18°C and every half hour when kept at 48°C to determine CFUs. Serial dilutions were made and three times 10 µl of each dilution were spotted onto Base II agar plates that were incubated for 2 days at 37°C under microaerobic conditions.

Biofilm assay was performed by Reeser et.al. (2007) with slight modifications. Stationary phase bacterial cultures were used to inoculate 1 ml MHB poured into separate wells of 24-wells polystyrene plate ((TPP®, Switzerland). For each bacterial culture the OD_{600} was adjusted to 0.25. Plates were incubated at 37°C microaerobically for 24, 48 and 72 h. Following incubation, the medium was removed, the wells were dried for 30 min at 55°C, and 1 ml of 0.1% crystal violet (CV) was added to each well for 30 min in room temperature. The unbound CV was removed and wells were washed two times with 1 ml distilled water. The wells were dried at 550C for 15 min and bound CV was decolorized with 200 µl 80% ethanol-20% acetone. One hundred microliters of this solution was removed from the wells and placed in 96-well plate, and the absorbance at 540 nm (*A*540) was determined using a microplate reader to determine biofilm formation. Control in all tests was included.

RESULTS AND DISCUSSION

Broiler fillets strains survived well at all temperatures, except 20°C, and were able to form biofilm at all time periods tested. Viability of post-disinfection strains was similar with broiler fillets strains, however they were less viable at 5°C. They also formed biofilm, yet after 24 hours of incubation they produced it most extensively compare to all strains tested. Interestingly all broiler fillets (except 348) and post-disinfection strains not only shared the ability to form biofilm but also were grouped together by there phylogenetic relationship. Meanwhile broiler cloacae strain found in broiler cloacae at farm and slaughterhouse failed to form biofilm and phylogenetically was similar to one of broiler fillets strains (348) which didn't form biofilm as well. Even though this strain was spread on the equipment surfaces and scalding water during slaughtering it was detected only on one broiler fillet sample at the end of slaughtering. We suppose that less viability at high temperature and inability to attach to the surface of meat might cause its low prevalence on broiler fillets.

Table 1. The origin and phylogenetical relationships of strains selected for survival at different temperatures and biofilm formation testing

Strains tested	Origin of strain	Phylogenetic roup
164	Broiler cloacae of slaughtered flock	2
348	Broiler fillets strain	2
330, 382, 266	Post-disinfection strains	1
283, 400, 353, 351, 349	Broiler fillets strains	1
508	Broiler cloacae persistent	3
140	Clinical strain	4

The phenotypic properties of two control strains (140, 508) were comparable to each other but differ from those obtained from the slaughtered broiler flock. In contrast to broiler fillets and post-disinfection strains both strains were more viable at 20°C, but lower viability was determined at -18°C and 48°C. However, same as broiler fillets and broiler cloacae strain from slaughterhouse they survived well at 5°C. In contrast to other strains differences between survival at 5°C and 20°C were not detected among control strains, thus suggesting that survival at 20°C is either the characteristic property required for persistence of *C. jejuni* strains in the environment either such property is specific to strains colonizing broiler or human gastrointestinal tract. Both strains could form biofilm only after 48 and 72 h, which shows that those strains adapt to adverse environmental contitions over time. The phenotypic differences between two broiler cloacae strains demonstrated in our study may be explained by the high genetic diversity of *C. jejuni* found in broiler cloacae at farm (Kudirkiene et. al., 2010) and they might differ in there ability to survive in the environment. Similar phenotypic properties found between clinical strain and persistent broiler cloacae strain reveal that there is a relationship between *C. jejuni* persistence and risk for human infection, thus encouraging us to focus on the control of such strains in the food chain and the environment.

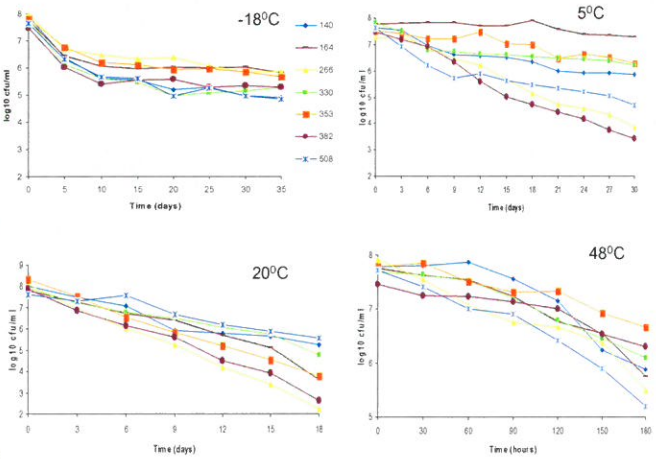

Fig. 1. Survival curves of *C. jejuni* strains. (164, 508) broiler cloacae strains; (140) clinical strain; (353) representative broiler fillet strain of median viability at particular temperature; (266, 382, 330) post-disinfection strains. Data shown are means from duplicate plates in a representative experiment.

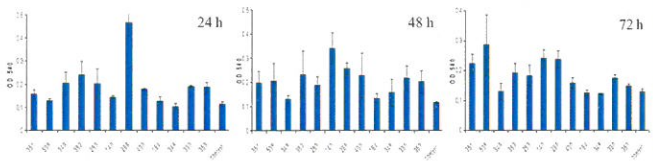

Fig. 2. The mean absorbance measured at 540 nm resulting from biofilm assay of *C. jejuni* strains grown in MH in 24-wells polystyrene plate at 37°C, microaerobically for different time periods. Biofilm formation was assessed by CV staining. Experiments were performed in triplicate on three separate occasions, and error bars represent one standart deviation from the mean.

References:

Birk, T., Ingmer, H., Andersen, M.T., Jørgensen K., Brøndsted, L., 2004. Chicken juice, a food-based model system suitable to study survival of *Campylobacter jejuni*. Letters in Applied Microbiology, 38 (1): 66-71.

Reeser, R.J., Medler, R.T., Billington, S.J., Jost, B.H., Joens, L.A., 2007. Characterization of *Campylobacter jejuni* biofilms under defined growth conditions. Applied and Environmental Microbiology. 73 (6): 1908-1913